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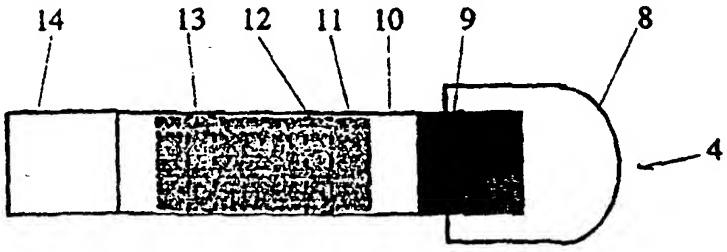
(57) Abstract



Assay device including a capsule, and housed within the capsule an assay insert made up of a filter unit, a conjugate reagent pad and a reaction membrane. The capsule has an opening covered by a capsule which is dissolvable by an external fluid containing an analyte. A porous filter unit occludes the opening in the capsule, providing for fluid communication of the analyte in the external fluid into the capsule when the capsule cap dissolves. The conjugate reagent pad housed in the capsule in fluid communication with the filter unit includes a mobilizable ligand with a label. The mobilizable ligand is capable of binding to the analyte to form a labelled, mobile ligand–analyte complex. A reaction membrane includes an immobilized detection reagent capable of binding the mobile ligand–analyte complex. The binding of the mobile ligand–analyte complex to the reaction membrane renders the label detectable on the membrane.

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ENCAPSULATED DIAGNOSTICS FOR ALIMENTARY ANALYTES

FIELD OF THE INVENTION

The invention is in the field of diagnostic assays, including immunological assays, for analytes in the alimentary canal, including gastrointestinal tract antigens.

BACKGROUND OF THE INVENTION

Standard immunological assays may be adapted for sensitive detection of a wide variety of antigens. Immunological assays in the form of test strips, for example, provide a medium in which the specific association of antigens and antibodies may be detected as the formation of one or more visible bands on a porous membrane. Such test strip devices have been adapted for a wide variety of qualitative and quantitative immunological assays, as described for example in the following documents: PCT Publication No. WO 97/37222 dated 9 October 1997; U.S. Patent No. 5,712,170 issued 27 January 1998; Lou *et al.*, 1993, Clin. Chem. 39(4):619-24; Riggin *et al.*, 1993, J. Chromatogr. 632(1-2):37-44; Birnbaum *et al.*, 1992, Anal. Biochem. 206(1):168-71; and, Klimov *et al.*, 1995, Clin.Chem. 41(9): 1360 (all of which are hereby incorporated by reference).

The alimentary canal, particularly the gastrointestinal tract, is a relatively harsh environment, in which proteins are typically degraded relatively rapidly, for example by proteolysis and denaturation. The low pH and high protease concentrations in the human stomach, for example, provide conditions that are not favorable for *in situ* immunoassays. In fact, conditions of low pH are commonly used to disrupt antigen-antibody binding, for example to elute antigens during affinity purification. Similarly, the environment of the small intestine in humans and other animals is not conducive to sensitive immunological testing *in situ*.

A standard method of extracting gastric fluids for analysis is gastroscopy, in which an endoscope is passed through the mouth of a patient into the gastrointestinal tract for sampling or biopsy. The discomfort caused by the procedure is however sufficient to make sedation or anesthesia desirable. A variety of approached have been developed in an effort to provide more convenient methods of sampling gastric

fluids. One approach has been to provide retrievable capsules with fibers adapted to capture gastric fluids, as for example are described in U.S. Patent No. 5,738,110 issued 14 April 1998 (which is hereby incorporated by reference). The purpose of such devices is typically to provide a sample for subsequent analysis, rather than providing a container in which an assay may be conducted and monitored.

SUMMARY OF THE INVENTION

The invention provides devices and methods for *in situ* immunological assays in the alimentary canal, including the gastrointestinal tract, or for *in vitro* immunological assays of alimentary or gastrointestinal fluids.

In one aspect, the invention provides an assay device including a capsule, and housed within the capsule an assay insert made up of a filter unit, a conjugate reagent pad and a reaction membrane, preferably further including a reservoir pad. The capsule has an opening covered by a capsule cap. The capsule cap is dissolvable by an external fluid containing an analyte. A porous filter unit housed in the capsule occludes the opening in the capsule, providing for fluid communication of the analyte in the external fluid into the capsule when the capsule cap dissolves. The porous filter unit may be provided with a facilitating reagent that is capable of mixing with the external fluid to provide a reaction condition inside the capsule different from conditions external to the capsule. A conjugate reagent pad is housed in the capsule in fluid communication with the filter unit. The conjugate reagent pad includes a mobilizable ligand with a label. The mobilizable ligand is capable of binding to the analyte to form a mobile ligand-analyte complex. A reaction membrane is housed in the capsule and is capable of fluid communication with the conjugate reagent pad, the reaction membrane includes an immobilized detection reagent capable of binding the mobile ligand-analyte complex. The binding of the mobile ligand-analyte complex to the reaction membrane renders the label detectable on the membrane. The assay device may also be provided with a reservoir pad in fluid communication with the reaction membrane.

The capsule cap may be made to be dissolvable in a gastrointestinal fluid,

such as gastric fluid or intestinal fluid. In various embodiments, the capsule cap material may be gelatin, cellulose-acetate phtalate, shellac or polyvinylpyrrolidone.

The mobilizable ligand with a label may be a labelled antibody, antibody fragment or antigen. Similarly, the immobilized detection reagent may be an antibody, antibody fragment or antigen. For example, antibodies may be used in embodiments where the analyte is an *H. pylori* antigen. Alternatively, antigens may be used where the analyte is an antibody.

In an alternative aspect, the invention provides diagnostic methods, particularly methods of using the capsule of the invention, in which a subject swallows the capsule and results are thereby obtained from an *in situ* assay conducted with the assay device of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is an isometric view of a capsule of the invention, showing the capsule cap fitted to the capsule body.

Figure 2 is a plan view of an assay insert of the invention housed in a capsule body.

Figure 3 is an isometric view showing an embodiment of the capsule body in which a portion of the capsule body may be separated from the remainder of the capsule body to facilitate particular uses of the assay of the invention.

Figure 4 is an isometric view showing alternative embodiments of the capsule body, in which portions of the capsule body are transparent.

Figure 5 is a plan view of an assay insert of the invention, showing zones on the reaction membrane that may be viewed to determine the assay results.

Figure 6 is a side elevational view of the assay insert of Figure 5, showing the parts of the assembled assay insert.

Figure 7 is a cross sectional view of an alternative embodiment of the assay device of the invention, showing a capsular embodiment containing dispersed assay components.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides devices and methods for assaying fluids in the alimentary tract of an animal for the presence of an analyte. As used herein, the term "analyte" includes any detectable molecules, particles, cells, cellular structures, proteins, glycoproteins, peptides, antibodies, antigens, small molecules, polysaccharides, drugs, toxins and equivalents thereof. Examples of particular analytes that may be of interest include whole or disrupted viral particles, such as cytomegalovirus, or viral proteins; bacteria, such as *Helicobacter pylori*, or bacterial proteins; fungi, such as *Candida albicans*, and fungal antigens; parasites, such as *Giardia lamblia* and *Cryptosporidia* or parasitic antigens; cancer marker such as alpha-feta-protein and carcino-embrionic-antigen.

In the assays of the present invention, ligands are used to detect analytes. Ligands are molecules that bind with high affinity to the analyte of interest. In some embodiments, ligands may be antibodies specific for the analyte, or antigen-binding fragments of an antibody. Alternatively, if the analyte is an antibody, the ligand may be an antigen to which the antibody of interest binds with high affinity. If the ligand is an antibody, it may be a monoclonal or polyclonal antibody, prepared in accordance with known techniques, such as affinity purified polyclonal antibodies, or purchased from commercial sources. In various embodiments of the invention, the ligands may be antibodies to *H. pylori, G. lamblia* or *Cryptosporidia* (such as may be available commercially from Biodesign International (ME, USA), CALTAG Laboratories (CA, USA) or Chemicon International, Inc. (CA, USA)).

Figure 1 illustrates a capsule that may be used in an embodiment of the invention, comprising a capsule body 2 and a capsule cap 3. The capsule cap 3 is sized to sealably engage the capsule body 2. The capsule body 2 and capsule cap 3 are sized to form a housing for an assay insert 4, when the capsule cap 3 is fitted to the capsule body 2. The capsule body is attached to a string 1, which may be used as described further herein to retrieve the assay device of the invention from the alimentary tract of a subject.

The string 1 for use in some embodiments of the assay device may be made of materials that are resistant to digestion, such as nylon line. Capsules may be provided

with strings of predetermined length optimized for particular applications. For example, capsules for paediatric use might be provided with 30 centimeters of string, while capsules for use with adult subjects might be provided with a string 60 cm long. The string may be provided with a loop or other retaining means at a position distal from the capsule, to facilitate retention of the string after a capsule is swallowed by a subject. The string is not required on embodiments of the assay device that are for *in vitro* use, or are to be swallowed and passed through a subject to be retrieved following defactory evacuation.

The capsule body 2 may be made of materials that are resistant to digestion in the gastrointestinal tract, such as polycarbonate. A resilient and rounded capsule body, as shown in several of the figures, may be provided to facilitate swallowing of the assay device. In some embodiments, the capsule body 2 and capsule cap 3 assembly may be sized to be similar to a conventional ingestible pharmaceutical capsule in size and shape.

In some embodiments, the capsule body 2 may be transparent. Alternatively the capsule body 2 may be provided with a transparent window, shown as 6 or 7 in Figures 4A and 4B. Preferably, any transparent window 6 or 7 in capsule body 2 is positioned to allow a reaction zone, shown as 11 in Figure 5, to be viewed through capsule body 2 (reaction zone 11 encompasses detection zone 12 and control zone 13, discussed in more detail below). Embodiments in which the capsule body 2 is entirely opaque may require the assay insert 4 to be removed from the capsule for analysis.

In alternative embodiments a portion of the capsule body may be weakened, as shown by dashed line 5 in Figure 3, to facilitate the removal of a part 20 of the capsule body 2 by hand or by using a tool. As described in more detail below, the removal of part 20 of the capsule body 2 may facilitate the use of the assay device of the invention for *in vitro* analysis of samples.

In alternative embodiments, the capsule body 2 and capsule cap 3 may be integral. In such embodiments, an equivalent structure for the assay device is provided by providing a portion of the capsule that is more dissolvable than the remainder of the capsule, wherein the more dissolvable portion may be fitted over the filter unit of

the assay insert. The term 'capsule cap' as used herein accordingly includes such portions of a capsule that are more dissolvable than other portions of a capsule.

The capsule cap 3 may be made of materials that maybe dissolved in the gastric fluid, such as gelatin. Such embodiments facilitate use of the assay device for detecting analytes in the stomach, where a gelatin cap will be dissolved after a relatively short period of contact with gastric fluids.

In alternative embodiments, the capsule cap 3 may be made of materials that are soluble in other portions of the gastrointestinal tract, such as the small intestine. In some such embodiments, the capsule cap may be made of materials such as cellulose-acetate phtalate, shelac or polyvinylpyrrolidone, that will be resistant to digestion until they reach the alkaline contents of the small intestine.

In various embodiments, the assay insert 4 may include a filter unit 8, a conjugate reagent pad 9, a reaction membrane 10, a reservoir pad 14 and a membrane backing 15. It will be appreciated by those skilled in the art that not all components are required in all embodiments, and equivalent structures may be substituted for the structures recited herein, when such equivalents are capable of functioning in the same way.

When the assay insert is installed in the capsule, the filter unit 8 occludes the opening in the capsule body 2. The filter unit thereby acts to physically separate the interior of the capsule from the exterior, while permitting fluid from the exterior to move into the interior of the capsule, so that analytes in the fluid may be subjected to the assay. The filter unit is preferably made of material that can absorb significant amounts of sample fluid from an external medium, and then release the sample fluid at a relatively steady controlled rate onto the remainder of the assay insert. The filter unit may also be provided with a facilitating reagent, such as a pH buffer, that interacts with the sample fluid so that the conditions of the fluid are adjusted to facilitate the interaction of the analyte and the assay ligands. The filter unit may thereby serve as both a physical and chemical gateway from the exterior of the assay device into the interior. Representative porous substances out of which the filter unit may be constructed include cellulose or glass fiber. The filter unit and the remainder of the immunological assay component will typically provided in a dry state, so that

they are capable of taking up moisture to bring analytes into contact with the immunological assay reagents, typically by capillary action.

In an embodiment where the capsule cap 3 is adapted to dissolve in a low pH environment, such as gastric juice, the filter unit may include a pH buffer adapted to raise the pH of the sample fluid. The buffer may for example be provided by infusing the filter unit with 200 mM TrisHCl, pH 8.5 and then drying the filter unit to leave it charged with buffer. In such an embodiment, the gastric fluid may be neutralized as it passes through the filter unit.

In embodiments in which the capsule cap 3 is adapted to dissolve in a relatively high pH environment, such as fluid from the small intestine, the filter unit may be provided with a pH buffer adapted to lower the pH of the sample fluid, such as 200 mM TrisHCl, pH 6.5. In such an embodiment, the intestinal fluid may be neutralized as it passes through the filter unit.

A wide variety of facilitating reagents may be used in the filter unit, alone or in combination. For example, protease inhibitors may be included in the filter unit where there are proteases in the sample fluid and it is desirable to inhibit the action of such enzymes in the interior of the assay device to facilitate ligand binding.

The filter unit 8 is in fluid communication with a conjugate reagent pad 9, so that fluid for analysis passes from filter unit 8 to conjugate reagent pad 9 once the capsule cap 3 breaks down. This movement of fluid may be driven by capillary action. The conjugate reagent pad may be made from porous, absorbent, relatively inert materials that mediate fluid flow by capillary action, such as fibrous material made from cellulose, cellulose nitrate, cellulose acetate or glass fiber.

The conjugate reagent pad includes a mobilizable ligand with a label. The mobilizable ligand is a moiety capable of binding to the analyte to form a mobile ligand-analyte complex bearing a label. The label may be conjugated to the mobilizable ligand covalently, or through other strong associations. Examples of some potentially useful labels include colloidal metal particles, such as gold particles; as well as colorimetric, fluorescent, and electrochemical particles made of latex or liposomes. A wide variety of labels may be chosen that are detectable when aggregated and that do not significantly interfere with ligand analyte binding. It will

be appreciated that some such labels are suitable for automated analysis, for example spectrophotometric analysis, for the purpose of detecting the label and, in some embodiments, automated quantitative analysis of the analyte concentration in the sample.

One or more mobilizable, labelled ligands may be included in the conjugate reagent pad, such as a plurality of antibody ligands conjugated to dyed-particles. In such embodiments, different ligands may be conjugated to distinguishable labels. For example, antibody ligands specific for different analytes may be labelled with dyed-particles of different colors, or florescent labels that emit at different wavelengths. Using this approach a single assay may be able to detect a number of different analytes.

The conjugate reagent pad 9 may be integral with filter unit 8, and need not be physically separate. For example, a single porous body 8, 9 may contain both a facilitating reagent and a mobilizable labelled ligand, so that the porous body serves as both the filter unit and the conjugate reagent pad. In such an embodiment, the conjugate reagent pad may be understood as a functional rather than a structural component of the assay device.

Conjugate reagent pad 9 is in fluid communication with a reaction membrane 10. Reaction membrane 10 includes a detection reagent capable of binding the mobile ligand-analyte complex, and not capable of binding the labelled ligand alone, so that the label is detectable on the reaction membrane 10 only when the analyte is present in the sample. Reaction membrane 10 may be made of a material that has sufficient porosity to allow movement of fluid samples containing the ligand-analyte complex by capillary action. Examples of materials that may be useful in some embodiments include fibrous material made of cellulose, cellulose nitrate, cellulose acetate or glass fiber membrane. Reaction membrane 10 may be provided in a variety of geometries and is by no means limited to an elongate strip conformation.

The immobilized detection reagent may be located on reaction membrane 10 in a defined detection zone, shown as 12 in Figure 5. The immobilized detection reagent binds to the analyte-ligand complex with sufficient affinity to immobilize the labelled ligand-analyte complex in the detection zone 12 under the assay conditions.

In this way, a detectable quantity of the label is aggregated in the detection zone 12 on reaction membrane 10 when the analyte is present in the sample. The immobilized detection reagent must not be capable of binding the labelled ligand to the detection zone 12 in the absence of the analyte. However, the immobilized detection reagent need not be specific only for the analyte, and could for example be specific for the combined analyte-ligand complex.

In some embodiments, the detection reagent may be an antibody directed against a different epitope on the analyte from the epitope recognized by the labelled ligand. When the analyte is an antibody, the detection reagent may be an antigen which binds to the analyte on the antigen-recognition site of the antibody, while the labelled ligand may be an antibody specific for the conserved portion of the antibody of interest.

A plurality of immobilized detection reagents may be included on a single reaction membrane, in one or more detection zones. One or more mobilizable labelled ligands may also be used, each being specific for a different kind of analyte. This combination of options may be utilized to provide assays for simultaneously obtaining data on the presence of a number of different analytes.

Reaction membrane 10 may also be provided with an immobilized control reagent that is capable of binding the labelled ligand in the absence of analyte. The control reagent may be immobilized on a distinct control zone, shown as 13 in Figure 5. The control zone 13 is preferably located on reaction membrane 10 further from filter unit 8 than detection zone 12, so that a detectable reaction at the control zone 13 is indicative that sample fluid has passed through reaction zone 12. The control reagent may therefore be useful to provide a positive control signal even in the absence of analyte, to confirm that the assay device is functional.

The assay insert 4 may be provided with a reservoir pad 14 in fluid communication with reaction membrane 10. Reservoir pad 14 may be made of an absorbent material, such as cellulose or glass fiber materials, capable of absorbing fluid transported along reaction membrane 10. Reservoir pad 14 may thereby be useful to facilitate transport of analyte through filter unit 8, conjugate reagent pad 9 and reaction membrane 10 by capillary action. Reservoir pad 14 is preferably located

downstream from detection and control zones 12, 13 so that it may facilitate fluid flow through such zones.

Assay insert 4 may be provided with a membrane backing 15, to physically support the assay insert. This may be useful, for example, to prevent the assay insert 4 from being deformed in the processes of swallowing and peristaltic movement associated with an *in situ* assay. Membrane backing 15 may be made of a plastic material, and bound to the reaction membrane 10 and the other components of the assay insert 4 by known means, such as adhesives or the process commonly called direct casting.

As shown in Figures 5 and 6, the assay of the present invention may be in the form of strip. Alternatively, the capsule of the invention may be occupied by three dimensional regions of materials that correspond to the functional regions of the strip, as shown in Figure 7, in which the following components are shown in cross section: capsule cap 3, filter unit 8, conjugate reagent pad 9, reaction membrane 10, detection zone 12, control zone 13 and reservoir pad 14. In such an embodiment, positive results in the detection and control zones 12, 13 would appear as circumferential bands of color or other indicator, visible through a transparent portion of the capsule body.

The invention provides an *in situ* diagnostic method, in which an assay device of the invention may be swallowed, typically with water and following a period of fasting. String 1 may be held by a subject, or taped to the subject, or otherwise tethered to adjust the distance that the assay device will travel down the alimentary tract. The capsule cap is adapted to break down when the assay device reaches the desired location in the tract, allowing sample fluid to pass through the filter and into the assay device. For example, the assay device may be tethered by string 1 so as to locate the device in the stomach of a subject, and the capsule cap adapted to dissolve in gastric juice. In such an embodiment, gastric juice moves by capillary action through filter unit 8, in which the fluid may be exposed to a buffering reagent, through the conjugate reagent pad 9, where an analyte-ligand complex forms if the analyte is present. The sample fluid then moves on to reaction membrane 10, where analyte-ligand complex travels to the detection zone, together with unbound labelled

mobilizable ligand. At the detection zone, the analyte-ligand complex is bound to the immobilized ligand, while uncomplexed labelled ligand remains unbound and continues to travel towards the control zone. At the control zone, the uncomplexed labelled ligand is bound to the control reagent.

Following an acceptable reaction time, the assay device may be retrieved from a subject for analysis using string 1. In some embodiments, reaction times of 30 minutes to 1 hour may be preferred for gastric juice assays, while reaction times of 3 to 4 hours may be preferred for assays in the small intestine.

If the label is visual, the results of an assay may be detected by viewing the detection zone and control zone through a transparent window 6, 7 in capsule body 2. If the label is detectable only at the control zone, the test for the analyte is negative. If the label is detectable at both the detection zone and the control zone, the test for the analyte is positive. If there is no detectable label at the control zone, the test may have failed for any of a variety of reasons.

In some embodiments, quantitative assays may be performed in accordance with the invention, in accordance for example with such methods of quantitative analysis for immunological assays as are described in PCT Publication No.. WO 97/37222 dated 9 October 1997 (incorporated herein by reference).

The components of the assay device are preferably non-toxic when the device is for use *in situ*. A wide variety of structural components with various physical properties may be selected for use in constructing alternative embodiments of the invention in accordance with the various functional requirements stated herein.

The following examples are merely illustrative of various embodiments of the invention, which is more generally defined in the claims.

EXAMPLE 1

The ability of a filter unit charged with a dried buffer to effectively neutralize an acid sample for immunological testing in a strip format was tested using a strip test for human chorionic gonadotropin (HCG, an immunological human pregnancy test) and a buffered solution (PBS buffer pH 7.2 or 2.0) containing HCG (at 1 IU/ml final concentration). Filter units were prepared of bonded polyester fiber dimensioned to

approximately 4mm x 7mm x 15mm and saturated with 100 µl or 200 µl of TrisHCl (1M pH 8.5). Following saturation with the buffer, the filter units were baked at 80°C for 15 minutes, then cooled to room temperature. The dried filter units charged with buffer were attached to commercially available test strips for HCG comprising a conjugate reagent pad, a reaction membrane and a reservoir pad. Test results were as follows. Test strips not provided with filter units were able to detect HCG in PBS at pH 7.2, but not in PBS at pH 2.0. Test strips provided with buffer-charged filter units were able to detect HCG both in PBS at pH 7.2 and at pH 2.0. The visibility of the positive bands for the samples tested at pH 2.0 was several orders of magnatude greater on the test strips provided with more buffer (200 µl of TrisHCl rather than 100 µl of TrisHCl).

EXAMPLE 2

To perform a test for *Helicobacter pylori* detection in the stomach, an assay device in capsule form may be swallowed with a half cup of water after an overnight fast. A string fastened to the capsule may held by tape to a subjects face, or held by hand. The capsule may be provided with a gelatin cap, which dissolves in the gastric juice once the capsule reaches the stomach by peristaltic movement. Once the capsule cap is dissolved, gastric fluid enters the capsule where it is first filtered and buffered by a cellulose mat filter unit provided with dried buffer. The buffer may for example be provided by infusing the filter with 200 mM TrisHCl, pH 8.5 prior to drying the filter. The sample fluid moves through the filter unit by capillary action and encounters the conjugate reagent pad, which contains polyclonal antibodies to an *H. pylori* antigen, the antibodies being conjugated to dyed-latex particles. Polyclonal *H. pylori* specific antibodies may be prepared, for example, in accordance with the methods taught in U.S. Patent No. 5,76,791 issued 10 February 1998, and incorporated herein by reference. The *H. pylori* antigens present in the fluid sample bind the dyed-particle conjugated antibodies to form a labelled antigen-antibody complex. The antigen-antibody complex moves with the sample fluid by capillary action towards the reaction zone of the reaction membrane. The detection zone on the

reaction membrane is provided with immobilized polyclonal antibodies against the same *H. pylori* antigen, so that the labelled antigen-antibody complexes are bound at the detection zone to the immobilized antibodies. Any unbound dyed-particle conjugated antibodies, as well as excess labelled antigen-antibody complexes continue to travel towards the control zone with the sample fluid by capillary action. At the control zone, the unbound dyed-particle conjugated antibodies bind to an immobilized antibody. The control zone antibody recognizes an epitope on the unbound dyed-particle conjugated antibodies.

After 30 minutes to 1 hour, the assay device capsule is retrieved using the string. The test results are then visualized through transparent portions of the capsule body. The presence of *H. pylori* antigens in the subjects gastric fluid is evidenced by the presence of two colored bands on the reaction zone, *i.e.* a visible band at the detection zone and a visible band at the control zone.

An alternative assay may be performed using the assay device of this example, wherein the gastric juice of the subject is retrieved using a gastrointestinal sampling device, such as an endoscope, and the gastric juice is assayed for *H. pylori* antigens by adding the assay capsule to the subjects gastric juice *in vitro*. To facilitate such an *in vitro* assay, the capsule cap may manually be removed, rather than allowing time for the capsule cap to dissolve in the gastric fluid. In further alternative embodiments, the capsule body may be breakable, as illustrated in Figure 3, to facilitate *in vitro* analysis. Also, the capsule may be retrieved following defecatory evacuation.

WHAT IS CLAIMED IS:

1. An assay device comprising:
   a. a capsule having an opening covered by a capsule cap, the capsule cap being dissolvable by an external fluid containing an analyte;
   b. a porous filter unit housed in the capsule occluding the opening in the capsule and providing for fluid communication of the analyte in the external fluid into the capsule when the capsule cap dissolves, the porous filter unit being provided with a facilitating reagent that is capable of mixing with the external fluid to provide a reaction condition inside the capsule different from conditions external to the capsule;
   c. a conjugate reagent pad housed in the capsule and capable of fluid communication with the filter unit, the conjugate reagent pad including a mobilizable ligand with a label, the mobilizable ligand being capable of binding to the analyte to form a mobile ligand-analyte complex;
   d. a reaction membrane housed in the capsule and capable of fluid communication with the conjugate reagent pad, the reaction membrane including an immobilized detection reagent capable of binding the mobile ligand-analyte complex so that the label is detectable on the reaction membrane.

2. The assay device of claim 1 further comprising a reservoir pad in fluid communication with the reaction membrane.

3. The assay device of claim 1 or 2 further comprising a string attached to the capsule adapted to permit retrieval of the capsule from the gastrointestinal tract of a patient.

4. The assay device of any on of claims 1 through 3 wherein the capsule cap is dissolvable in a gastrointestinal fluid.

5. The assay device of any one of claims 1 through 4 wherein the capsule cap is dissolvable in a gastric fluid.

6. The assay device of claim 5 wherein the capsule cap is comprised of a gelatin material dissolvable in the gastric fluid.

7. The assay device of any one of claims 1 through 4 wherein the capsule cap is dissolvable in an intestinal fluid.

8. The assay device of claim 7 wherein the capsule cap is comprised of a material selected from the group consisting of cellulose-acetate phtalate, shellac or polyvinylpyrrolidone.

9. The assay device of any one of claims 1 through 8 wherein the mobilizable ligand with a label is a labelled antibody or antibody fragment.

10. The assay device of any one of claims 1 through 8 wherein the analyte is an antibody and the mobilizable ligand is an antigen bound by the antibody.

11. The assay device of any one of claims 1 through 10 wherein the immobilized detection reagent is an antibody or antibody fragment.

12. The assay device of any one of claims 1 through 11 wherein the analyte is an antibody and the immobilized detection reagent is an antigen bound by the antibody.

13. The assay device of any one of claim 1 through 12 wherein the reaction membrane comprises an immobilized control reagent that is capable of binding the mobilizable ligand with a label in the absence of analyte.

14. The assay device of any one of claims 1 through 9, 11, or 13 wherein the analyte is an *H. pylori* antigen.

15. The assay device of any one of claims 1 through 9, 11, or 13 wherein the analyte is selected from the group consisting of whole or disrupted viral particles, cytomegalovirus particles, viral proteins, bacteria, bacterial proteins, fungi, *Candida albicans*, fungal antigens, parasites, *Giardia lamblia, Cryptosporidia,* parasitic antigens, cancer markers, alpha-feta-protein, and carcino-embrionic-antigen.

16. The assay device of any one of claims 1 through 15 further comprising a string attached to the capsule, wherein the string is sized to permit retrieval of the capsule from a patient.

17. The assay device of any one of claims 1 through 16 wherein the capsule further comprises a transparent window adapted for visualizing the reaction membrane.

18. A method of detecting an analyte in a subject, comprising:

a. providing a swallowable capsule having:

i. an opening covered by a capsule cap, the capsule cap being dissolvable by an alimentary fluid containing an analyte;

ii. a porous filter unit housed in the capsule occluding the opening in the capsule and providing for fluid communication of the analyte in the alimentary fluid into the capsule when the capsule cap

dissolves, the porous filter unit being provided with a facilitating reagent that is capable of mixing with the alimentary fluid to provide a reaction condition inside the capsule different from conditions external to the capsule;

iii. a conjugate reagent pad housed in the capsule and capable of fluid communication with the filter unit, the conjugate reagent pad including a mobilizable ligand with a label, the mobilizable ligand being capable of binding to the analyte to form a mobile ligand-analyte complex;

iv. a membrane housed in the capsule and capable of fluid communication with the conjugate reagent pad, the membrane including an immobilized detection reagent capable of binding the mobile ligand-analyte complex so that the label is detectable on the membrane;

b. allowing the subject to swallow the capsule;

c. allowing the capsule to be exposed to the alimentary fluid of the subject for a period of time;

d. detecting the label on the membrane.

19. The diagnostic method of claim 18 wherein the assay device further comprises a reservoir pad in fluid communication with the membrane.

20. The diagnostic method of claim 18 or 19 wherein the assay device further comprises a string attached to the capsule adapted to permit retrieval of the capsule from the gastrointestinal tract of a patient, and the method further comprises the step of retrieving the capsule using the string prior to detecting the label on the membrane.

21. The diagnostic method of any one of claims 18 through 20 wherein the capsule cap is dissolvable in a gastrointestinal fluid.

22. The diagnostic method of any one of claims 18 through 20 wherein the capsule cap is dissolvable in a gastric fluid.

23. The diagnostic method of any one of claims 18 through 20 wherein the capsule cap is comprised of a gelatin material dissolvable in a gastric fluid.

24. The diagnostic method of any one of claims 18 through 20 wherein the capsule cap is dissolvable in an intestinal fluid.

25. The diagnostic method of any one of claims 18 through 20 wherein the capsule cap is comprised of a material dissolvable in an intestinal fluid, wherein the material is selected from the group consisting of cellulose-acetate phtalate, shellac or polyvinylpyrrolidone.

26. The diagnostic method of any one of claims 18 through 25 wherein the mobilizable ligand with a label is a labelled antibody or antibody fragment.

27. The diagnostic method of any one of claims 18 through 25 wherein the analyte is an analyte antibody and the mobilizable ligand is an antigen bound by the analyte antibody.

28. The diagnostic method of any one of claims 18 through 27 wherein the immobilized detection reagent is an antibody or antibody fragment.

29. The diagnostic method of any one of claims 18 through 27 wherein the

analyte is an analyte antibody and the immobilized detection reagent is an antigen bound by the analyte antibody.

30. The diagnostic method of any one of claims 18 through 26 or 28 wherein the analyte is an *H. pylori* antigen.

31. The diagnostic method of any one of claims 18 through 26 or 28 wherein the analyte is selected from the group consisting of whole or disrupted viral particles, cytomegalovirus particles, viral proteins, bacteria, bacterial proteins, fungi, *Candida albicans*, fungal antigens, parasites, *Giardia lamblia, Cryptosporidia,* parasitic antigens, cancer markers, alpha-feta-protein, and carcino-embrionic-antigen.

32. The diagnostic method of any one of claims 18 through 31 wherein the capsule is retrieved for detection following defacatory evacuation.

33. The diagnostic method of any one of claims 18 through 31 wherein the reaction membrane of the assay device further comprises an immobilized control reagent that is capable of binding the mobilizable ligand with a label in the absence of analyte, and the step of detecting comprises detecting the label on the membrane on the immobilized control reagent.

34. The diagnostic method of any one of claims 18 through 33 wherein assay device is provided with a transparent window and the step of detecting comprises visualizing the label through the transparent window.

35. The diagnostic method of any one of claims 18 through 33 wherein the step of detection comprises an automated analysis.

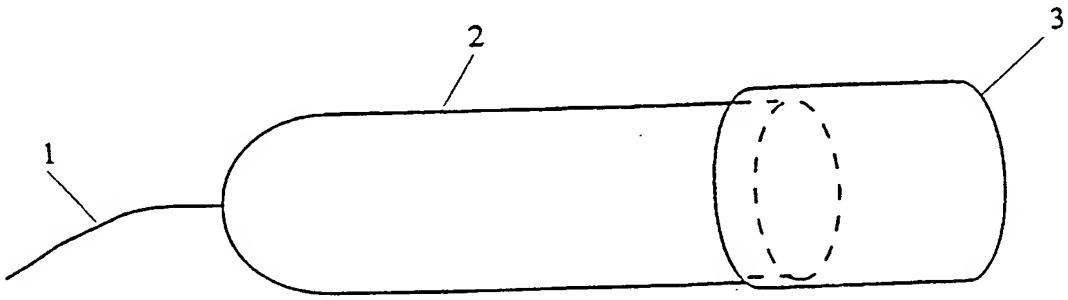


Figure 1

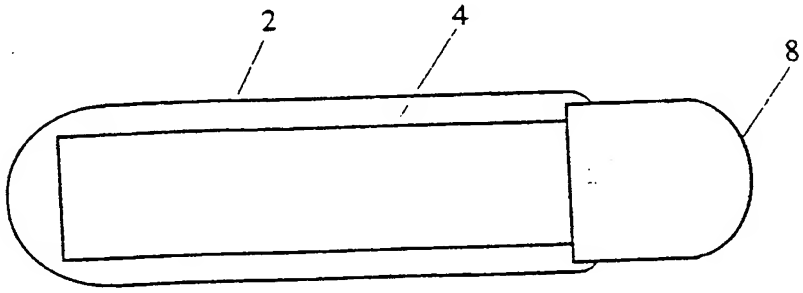


Figure 2

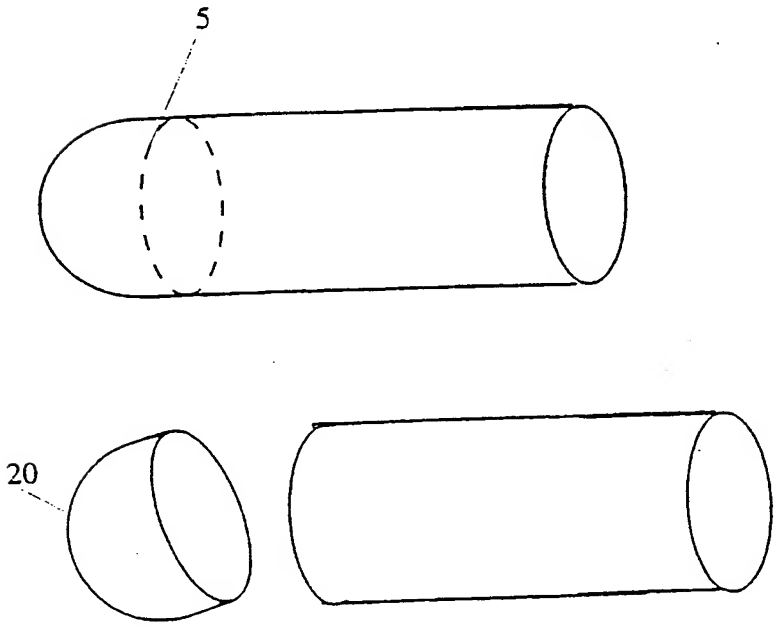


Figure 3

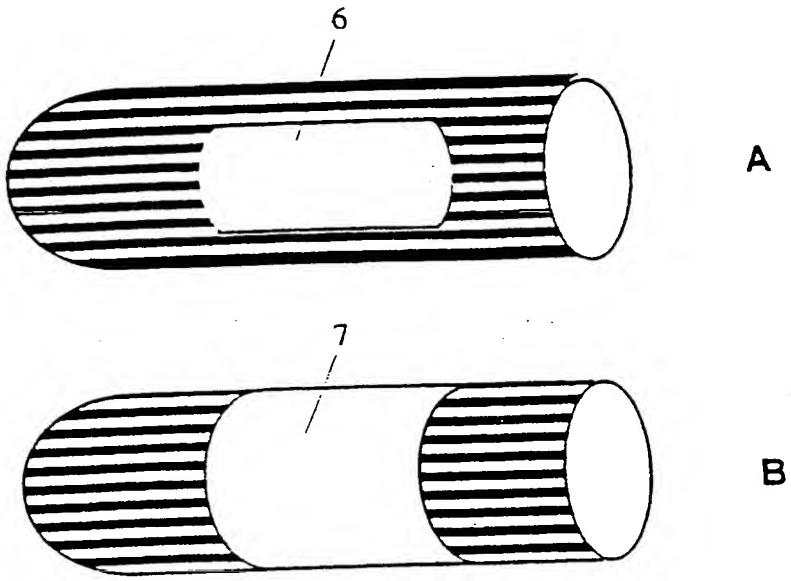


Figure 4

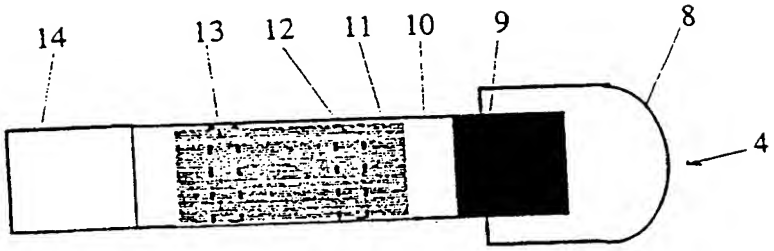


Figure 5

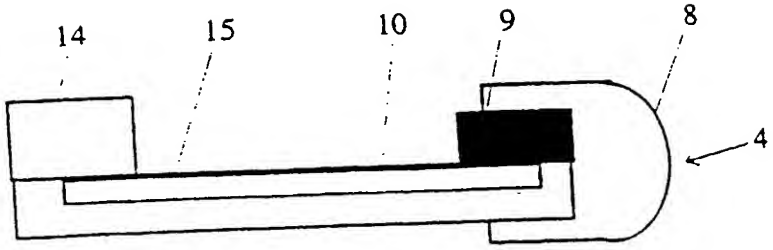
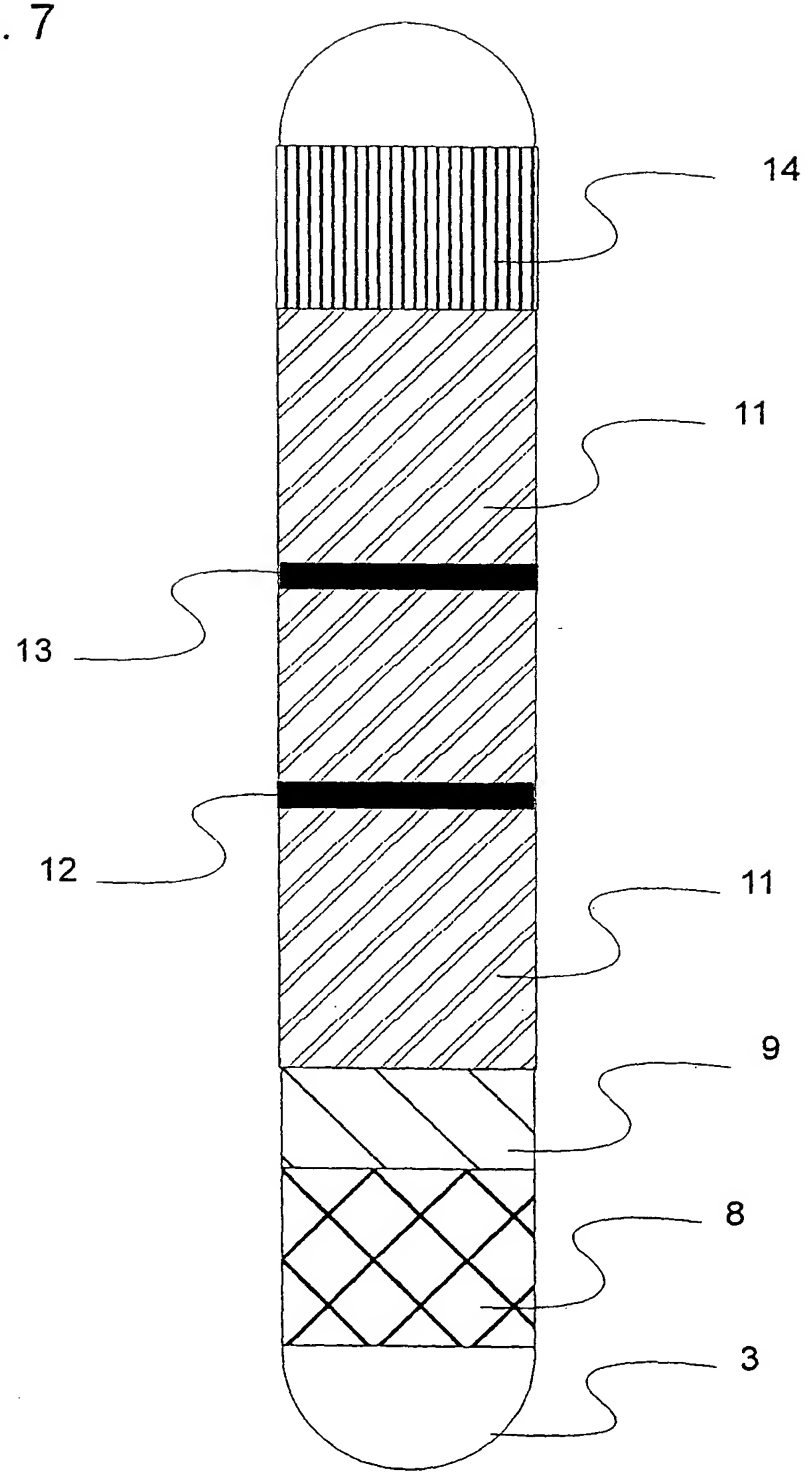


Figure 6

Fig. 7



INTERNATIONAL SEARCH REPORT

International Application No
/CA 99/00172

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/558 G01N33/569

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N C12Q A61B A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	US 5 738 110 A (HUGHES MARK A ET AL) 14 April 1998 cited in the application see the whole document ---	1-35
A	US 5 716 791 A (KOZAK KENNETH JAMES ET AL) 10 February 1998 cited in the application see claim 1 ---	1-35
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A	WO 95 11672 A (MARSHALL BARRY) 4 May 1995 see page 6, line 23, paragraph 2 - page 8, line 16, paragraph 3 ---	1-35

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
30 June 1999	09/07/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hart-Davis, J

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INTERNATIONAL SEARCH REPORT

International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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